Two yeast/*Escherichia coli* λ/plasmid vectors designed for yeast one- and two-hybrid screens that allow directional cDNA cloning

Johan Memelink

Institute of Molecular Plant Sciences, Clusius Laboratory, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

Keywords: Gene expression, Cloning & sequencing

▼Two yeast/*Escherichia coli* λ/plasmid vectors with a number of attractive features are described here. They allow unidirectional cloning of ZAP-synthesized cDNAs of up to 10 kb in size. They use the *cre-lox* system for easy, efficient excision of plasmids that replicate at high copy number both in yeast and *E. coli*. The plasmid vectors allow a choice of two yeast selectable markers, URA3 and LEU2, as well as alternative yeast promoters. They direct the constitutive expression of fusions between the GAL4 transcriptional activation domain II (AD) and the cDNA-encoded protein in yeast, and therefore allow cloning of protein interaction partners in two-hybrid screens (Ref. 1), or of DNA-binding proteins in one-hybrid screens (Ref. 2).

Unidirectional cDNA libraries increase the efficiency of expression-based screening twofold. The ZAP cDNA synthesis kit (Stratagene) is a reliable and popular system for generating full-length cDNAs which can be cloned unidirectionally due to the presence of a 5′ *Eco*RI sticky end and a 3′ *Xho*I sticky end. A number of yeast/*E. coli* plasmid shuttle vectors have been described that enable unidirectional cloning of ZAP cDNA fused with GAL4 (e.g. vectors available from Clontech). However, the generation of large, representative libraries in a plasmid vector is technically demanding and tends to select for smaller cDNA inserts. Cloning in λ vectors, on the other hand, enables efficient production of very large libraries, and is not size-selective. Only one λ vector has been described to date that enables unidirectional cloning of cDNAs in a fusion with the GAL4 AD [HybriZAP (Stratagene)]. It is converted to a yeast/*E. coli* shuttle plasmid using the f1 initiator/terminator *in vivo* excision system (Stratagene).

Here, two other λ vectors that offer similar features are described. The λACTII vector (Fig. 1) was generated by cloning the plasmid pACTII in λYES (Ref. 3) arms digested with *Not*I. pACTII is very similar to pACT2 (Clontech; Genbank no. U29899), and contains the same ADH1 promoter, polylinker and terminator sequences. λACTII is very similar to the bidirectional cloning vector λACT (Ref. 4). The second vector, λYPG (Fig. 1), was constructed by cloning a GAL4 AD fragment from pACTII, generated with *Hin*dIII (made blunt-ended with the Klenow fragment of *E. coli* DNA polymerase I) and *Eco*RI, in pYPGE15 (Ref. 5) digested with *Sma*I and *Eco*RI, resulting in pYPGE15-GAD, and introducing the latter plasmid in λYES arms using *Not*I. Unique *Eco*RI and *Xho*I restriction sites in the polylinker make these λ vectors compatible for unidirectional cloning of ZAP-synthesized cDNAs of up to 10 kb in size. The λ clones can be converted to yeast/*E. coli* shuttle plasmids by *in vivo* recombination of the *lox* sites in a CRE-expressing *E. coli* host strain. Any λ-sensitive *E. coli* can be converted into a CRE-expressing host by lysogeny with the *cre*-containing λKC (Ref. 3). *cre-lox* excision is somewhat more reproducible and efficient than f1 initiator/terminator-mediated excision. *In vivo* excision results in plasmids lacking one of the two *lox* sites and the *Not*I site, termed pACTII′ and pYPGE15-GAD′, that contain the ColE1 origin and ampicillin resistance marker for replication and selection in *E. coli*, and the 2 μ origin and the LEU2 and URA3 genes, respectively, for maintenance in yeast. pACTII′ and pYPGE15-GAD′ carry the constitutive ADH1 and PGK promoters, respectively, which direct the expression of fusion proteins

Corresponding author: memelink@rulsfb.leidenuniv.nl

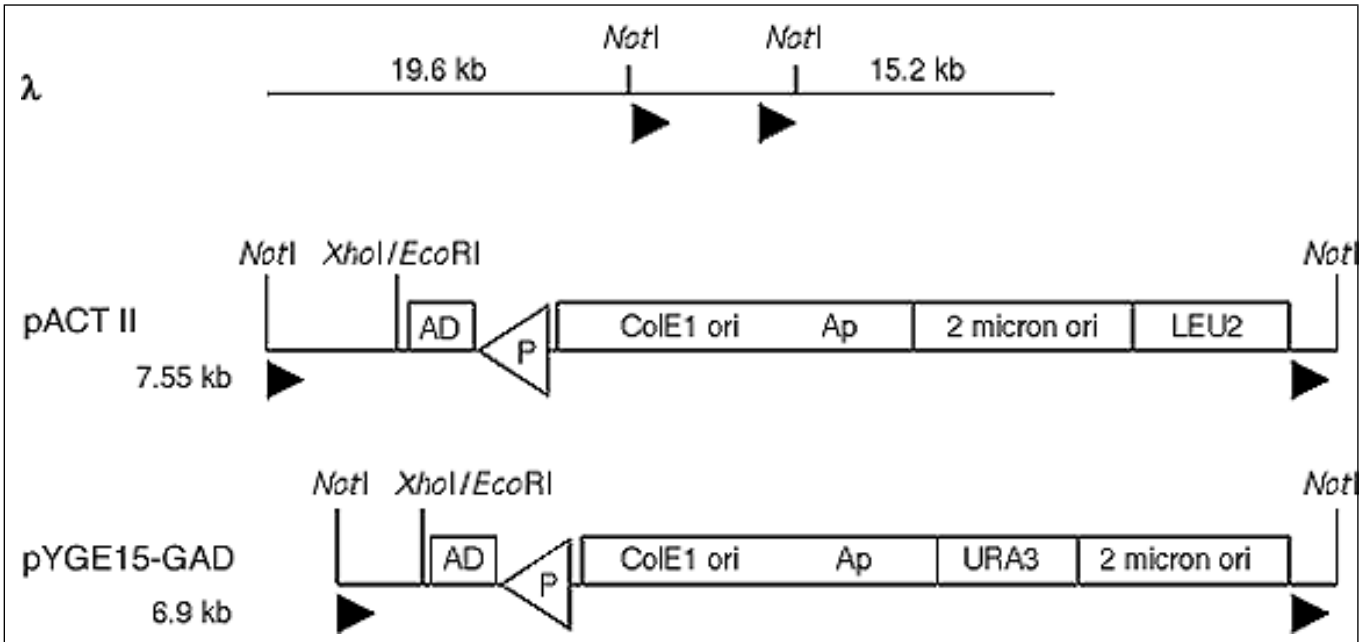

FIGURE 1. Map of yeast/*E. coli* λ/plasmid vectors for unidirectional cDNA cloning. pACTII and pYPGE15-GAD were cloned in *Not*I-digested λYES arms in the orientation indicated. pACTII carries the ADH1 promoter and pYPGE15-GAD the PGK promoter (P) to drive the constitutive expression of fusions between the GAL4 activation domain (AD) and cDNA-encoded proteins cloned in the *Eco*RI and *Xho*I sites. *lox* sites are indicated with black triangles. CRE-mediated *in vivo* excision results in circular yeast/*E. coli* shuttle vectors that lack the *Not*I site and one of the *lox* sites. Ap, Ampicillin resistance gene; ori, origin of replication.

of the nuclear localization sequence of SV40 T antigen, amino acid residues 768–881 of GAL4, the hemagglutinin (HA) epitope and the cDNA-encoded protein (Ref. 4). I have generated very large (>2 × 10^6 independent clones) cDNA libraries of the plant species tobacco, *Arabidopsis* and periwinkle in these vectors using the ZAP cDNA synthesis kit under the standard conditions recommended by the manufacturer. As reported previously (Ref. 5), efficiency of *in vivo* excision is about 50% colony formation per infecting phage titer. Using the periwinkle plasmid library, different classes of DNA-binding proteins have been cloned in one-hybrid screens by my group (unpublished results).

References

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Products Used

ZAP cDNA synthesis kit: ZAP cDNA synthesis kit from Stratagene
cDNA: cDNA from Clontech Inc
HybriZAP: HybriZAP from Stratagene
pACT2: pACT2 from Clontech Inc